

Policy & Procedure (P&P)

Policy Title :		
ANTIBODY IDENTIFICATION		
Department	Index No.	Scope
Laboratory & Blood Bank	LAB-165	Staff: All Blood Bank Staff
Issue Date	Revision NO	Effective Date
1432/6/7	NO.: 3	1441/7/19
Review Due Date	Related Standard NO.	Page Number#
1443/7/19	CBAHI (61)	Page 1 of 8

01. Policy:

The blood bank performs the antibody identification for each positive antibody screening.

02. Definition :

Antibody: An immunoglobulin, a specialized immune protein, produced because of the introduction of an antigen into the body

03. Purpose :

The antibody identification is performed to determine if the patient has developed allo-antibodies and helps us to choose the right blood component for cross matching.

04. Procedure :

The Antibody identification can be performed by the following methods

- A) Tube method.
- B) column technology & the Gel Microtyping system.
- C) *Tango infinity (Fully automated machine).*

Antibody identification is done in the following conditions:

- 1-A panel of RBCs of known antigenic configuration is used to identify any antibody detected in the patient serum by:
 - a) Positive antibody screen.



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- b) Incompatible cross match.
- c) Elution from red cells which give positive DAT.
- 2-Antibody identification helps in:
 - a) Pretransfusion testing to select antigen negative blood for transfusion.
 - b) Prenatal testing to predict the type of HDN.
- 3-The serum or elution is tested at all test phases.

A) Tube method technique

1-Specimen Requirements

Fresh serum obtained from fully clotted specimens should be used in antibody identification procedures. The use of plasma is not recommended since anticoagulants may interfere with the detection of complement binding antibodies. Fibrin clots may also interfere. Serum should be separated from the red cells immediately and testing should be performed as soon as possible. If testing is delayed, the serum should be stored at 2-8°C for no more than 48 hours or frozen.

Hemolyzed specimens are unacceptable for antibody identification procedures because some antigen antibody reactions are detected by visible hemolysis in the test tube.

2-Materials

- 2.1 Glass test tubes .
- 2.2 Pasteur pipettes .
- 2.3 Isotonic saline 0.85% NaCl.
- 2.4 Calibrated serologic centrifuge.
- 2.5 37°C water bath .
- 2.6 Timer.
- 2.7 Microscope and slides.

3-Reagents

- 3.1 Commercially prepared panel of all group O reagent red cells (must use same lot numbers as a matched set)
- 3.2 20% bovine albumin
- 3.3 Antihuman globulin (Coombs reagent).
- 3.4 Coombs control cells(CCC).

4-Method

4.1 Saline Phase:

- 4.1.1 Label one test tube for each panel cell to be used and one additional tube for an autologous control.
- 4.1.2 Place 2-3 drops of serum to be tested into each of the tubes; (increasing the amount of serum may help to detect weak reactions caused by weak antibodies).
- 4.1.3 Completely resuspend all panel cells by gently inverting each vial several times.
- 4.1.4 Add one drop of each reagent panel cell to the appropriately labelled tubes. Mix each tube thoroughly and Centrifuge .
- 4.1.5 Examine supernatants for hemolysis and gently resuspend each cell button and examine for agglutination against a well-lighted background.



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4.2 Albumin Phase:

- 4.2.1 Add two drops of 22% bovine albumin.
- 4.2.2 Mix and incubate at 37° C for 20-30 minutes. Weak reacting antibodies are easier detected when incubating 60 minutes.
- 4.2.3 Mix and centrifuge.
- 4.2.4 Examine for hemolysis and gently suspend and observe for agglutination. Record results.

4.3 Antiglobuline phase:

- 4.3.1 Wash the cells with the isotonic saline three times
- 4.3.2 Add two drops of anti-human serum.
- 4.3.3 Mix and centrifuge .
- 4.3.4 Examine for agglutination macroscopically and microscopically. Record results.

- 5 Control all negative tests by adding one drop of coombs control cells. These cells must agglutinate the negative test to be valid.

6 Quality Control:

Negative or weakly positive reactions obtained in the antiglobulin phase of testing should be confirmed using red cells sensitized with IgG. Those tubes which give a negative or less than 1+ reaction with this reagent should be repeated. In addition to a visual check for deterioration of the reagent panel cells, their reactivity may be checked periodically by testing antigens likely to deteriorate, such as le, M. Fya, with a weakly reactive antibody of the same specificity. If such cells are found non-reactive the panel should not be used.

7 Reporting Results:

7.1-Interpretation

- 7.1.1 Agglutination of any panel cell at any phase or hemolysis at the saline or albumin phase of testing constitutes a positive test and indicates the presence of antibody(ies) in the serum to antigen(s) in that reagent cell. No agglutination or hemolysis throughout the procedure of any cell indicates that the serum does not contain detectable antibodies to any of the antigens present in the reagent cells by the technique used.

- 7.1.2 The following criteria should be used to properly interpret the identification of an unknown antibody.

- 7.1.2.1 Review the reactions obtained with the autocontrol , if test cells are positive&autocontrol is negative this indicate alloantibody only, but if test cells are positive&autocontrol is also positive this indicate autoantibodies& may be alloantibody also

- 7.1.2.2 Delete all antigens present on those red cells shown to be non-reactive at all phases at testing by drawing a slash(Roll out). At the end of this process, any antigen with two or more hash marks through to be considered tentatively "ruled out".

Results obtained on the Antibody Screen should be included in the process.

- 7.1.3 To "rule in" or confirm the suspected antibody specificity:

- 7.1.3.1 The serum must react negatively with two or preferably three cells negative for the antigen, and react positively with two or preferably three cells that are positive

for the antigen(Rule of three).

7.1.3.2 Observe the overall reactivity of the antibody or antibodies for any patterns that may assist in the identification process(Matching pattern)..

7.1.3.3 Lastly, the patient must be phenotyped to the antigen specific to the suspected antibody& it must negative for it to consider it the causative antibody.

7.1.4. Circle all remaining antibody specificities that have not been ruled out (antigens that have zero or one hash mark through them).

7.1.5 Compare the pattern of agglutinated cells with the antigen profiles not deleted from the master list(Matching pattern).

7.1.6 If only one antigen remains after deleting those antigens present on all non-reactive panel cells, and the patterns of the antigen matches the pattern of reactivity obtained, the specificity of the antibody is tentatively identified, patient red cells must be tested with the appropriate antiserum to ensure that the patient lacks this antigen.

7.1.7 If more than one antigen remains following the deletion process then steps must be taken to identify the multiple antibodies which may be present; i.e. panel studies with enzyme phase included or use selected cells (every cell should be positive for one of the antibodies and negative for the remaining antibodies)

7.1.8 Positive or negative results that do not fit any of the established antigen patterns may indicate the presence of the multiple antibodies or antibodies to unspecified antigens.

7.1.9 If it is suspected that multiple antibodies are present, it is appropriate to perform another panel using reagent cells of another manufacturer in order to possibly delete additional antigens from the prospects. If multiple antibodies are still suspected, review the phase(s) and strength of the reactions obtained. The pattern of reactions at each test phase, when each phase is considered independently may match the profile of an antigen on the master list, thus giving a clue to the specificity of at least one of the antibodies that might be present. Test the patient's own red cells for antigen corresponding to suspected antibodies present. If the patient's red cells possess the antigen, it is unlikely that the antibody is present unless an autoantibody is suspected.

2. Recording of Results:

7.2.1 Upon identification of suspected antibody(ies) the results should be clearly recorded on the panel worksheet along with the patient's name, identification number and blood grouping.

7.2.2 The results of any antibody identification shall also be noted on the blood bank worksheet in the comments section opposite the patient's group and the antibody screen.

3. Reporting of Results:

The results of an antibody identification should be reported on the original requisition form if the antibody corresponds to a specific antigen, such as "Anti-Le". If the identification is inconclusive, technologist should report "Identification inconclusive; request additional serum for further studies".

4. Limitations:

Falsely positive or falsely negative results can occur from bacterial or chemical contamination of tests materials, inadequate incubation time or temperature, improper centrifugation, improper storage of test materials or if an inappropriate serum-to-cell ratio is used.

7.4.1 Most commercial panel cells possess only the most commonly inherited red cell antigens. It is



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- possible that a particular serum will contain an antibody defining an antigen that is not present on these cells (e.g. low incidence antibodies).
- 7.4.2 Negative reactions will be obtained if antibodies are present in concentrations too low to be detected by the test method.
- 7.4.3 Some antibody specificities may not be ruled out because they are so rare that none of the cells on the panel are positive for them, (Low incidence antibody) or one cell may be positive but doesn't appear to be the cause of the reactions.
These antibody specificities may be "tentatively" ruled out (usually f, C^w, V, Kp^a, Js^a, and Lu^a).
- 7.4.4 Be aware that dosage can occur causing antigens to be erroneously excluded { the Rh, Duffy (Fy^a, Fy^b), MN and Kidd (Jk^a, Jk^b) S s systems are dosage}. It is important to consider the strength of antigen expression & the phase of reaction.
- 7.5 Landsteiners rule: Individuals **DO NOT** make allo-antibodies against antigens they have

B) Gel Micro typing System

1- Materials

- 1.1 ID-Centrifuge
- 1.2 ID-Incubator 37°C
- 1.3 ID-Working table
- 1.4 ID-Dispenser
- 1.5 ID-Pipette FP-4
- 1.6 ID-Disposable tips

2 Reagents

- 2.1 ID-Diluent 2(modified LISS).
- 2.2 ID-DiaCell panel (Antibody identification cell "1-11").

3-Micro typing cards

LISS/ Coombs

4-Sample

Serum or plasma.

5-Test procedure

- 5.1 Allow all reagents to reach room temperature before use.
- 5.2 Identify the ID- micro typing card with the patient name and number. Remove the aluminum foil.
- 5.3 Add 50µl of ID-DiaCell panel to micro tube "1-11".
- 5.4 Add 25µl of test serum or plasma to each micro tubes.
- 5.5 Incubate the micro typing card for 15 minutes at 37°C in the ID-incubator.
- 5.6 Centrifuge the micro typing card for 10 minutes in the ID-centrifuge.
- 5.7 Interpret the result.



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6-Interpretation

Positive reaction grade 1-4 in the micro tube indicates the presence of an antibody. Negative reactions in the micro tubes indicates the absence of an antibody. procedure.

C)Antibody identification using TANGO INFINITY System(Fully automated machine)

1-Principle of the test

- The Solidscreen™ II Strip plates are composed of test strips (12 strips with 8 wells each) that have been coated with Protein A.
- Protein A has a high binding affinity for the Fc region of immunoglobulins
- The method used for the antibody screen test on the Solidscreen™ II Strip is solid phase adherence.
- If red blood cell alloantibodies and/or autoantibodies are present in the sample to be tested they will bind to the identification red blood cells.
- Unbound antibody is removed by washing.
- After adding Anti-Human- Globulin specially formulated for the Solidscreen™ II assay and centrifugation Anti-Human-Globulin forms a link between the protein A on the surface of the microwell and the identification red blood cells. Thereby a layer of red blood cells will form on the surface of the microwell (positive for antibody identification).
- But if no red blood cell alloantibodies or autoantibodies are present in the sample, the cells will not get coated with antibodies and therefore will not form a cell layer on the surface of the microwell but sediment to the bottom of the well to form a cell button

2-Sample

Serum or plasma after proper centrifugation.

3-Reagents

- 3.1 Solidscreen™ II Strip Plates with 12 strips per plate. The strips are coated with protein A.
- 3.2 MLB 2 Modified LISS 2 (Low Ionic Strength Solution) is an antibody enhancement media specifically formulated for use with the Solidscreen™ II test system. The MLB 2 is used in the preparation of a 1% cell suspension of sample red blood cells.
- 3.3 Alsevers Solution Used as a suspension medium for red blood cells.
- 3.4 Anti-Human Globulin (Anti-IgG Solidscreen™ II) The Anti-Human Globulin is used to demonstrate the presence of antibodies bound to the donors red blood cell that do not cause direct agglutination.
- 3.5 Antibody identification panel

4-Materials:

- 4.1 Glass test tubes.
- 4.2 X rack of the Tango Infinity machine.
- 4.3 Calibrated serologic centrifuge



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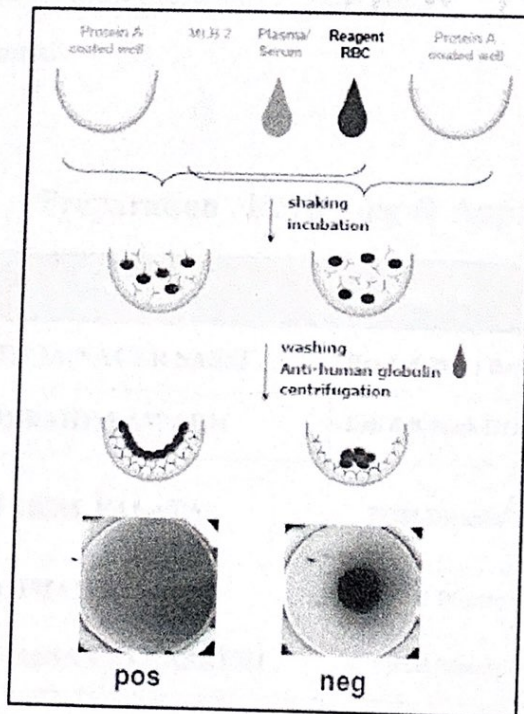
5- **Test procedure:**

- 5.1 Centrifuge Patients or donor blood sample & put them in (N) Rack.
- 5.2 Open the 11 vials of the panel & put them in reagent Rack
- 5.3 Open the sample door & put the (N) Rack in its correct place.
- 5.4 Push the Rack button to select the desired rack on the screen.
- 5.5 Press manual entry button & write the ID number of patients or donors sample.
- 5.6 Press accepted entry button
- 5.7 Press (ABSd11) button to select the test & close the sample door.
- 5.8 Press start button to start the test.
- 5.9 After the end of the test validate the results.
- 5.10 Print out the results.
- 5.11 Record the results in the special logbook

6. **Quality Control:**

Control Set QC contain test erythrocytes with defined antigen pattern for ABO, Rh(D), phenotyping (Rh(CEce) and Kell) as well as isoagglutinins for reverse typing,

- 6.1 Group O Neg
- 6.2 Group AB Pos
- 6.3 Group A neg
- 6.4 Group O Pos
- 6.5 Solidscreen 11-Control is used for Antibody screen QC



05. Responsibilities :

All Laboratory Staff of Al-Qunfudah General Hospital.

06. Equipment & Forms

Daily Reagent Quality Control

07. Attachment :

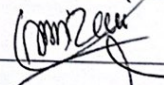
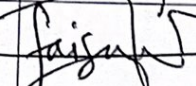
N.A

08. Reference

Technical Manual of the American Association of Blood Banks

TANGO INFINITY manual

Preparation , Reviewing & Approval Box

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